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14. ABSTRACT Studies performed under this grant provided evidence that tissue injury and hypoperfusion activate systemic and central neural, hormonal and opiate mechanisms that regulate the hemodynamic, metabolic and proinflammatory counter regulatory responses involved in restoring homeostasis following trauma/hemorrhage. Opiate pathway activation favors hemodynamic instability and a pro-inflammatory tissue response while sympathetic nervous system activation counteracts the inflammatory response and contributes to cardiovascular responsiveness. Our studies demonstrated that the intact neuroendocrine response is critical to ensure survival and host defense mechanisms from secondary infectious challenges. Furthermore, stress-and analgesia-induced disruptions in response exacerbate hemodynamic instability, compromise tissue perfusion and predispose to tissue injury and impaired innate host-defense response to a subsequent challenge.					
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Studies performed under this grant provided evidence that tissue injury and hypoperfusion activate systemic and central neural, hormonal and opiate mechanisms that regulate the hemodynamic, metabolic and proinflammatory counter regulatory responses involved in restoring homeostasis following trauma/hemorrhage. Opiate pathway activation favors hemodynamic instability and a pro-inflammatory tissue response while sympathetic nervous system activation counteracts the inflammatory response and contributes to cardiovascular responsiveness. Our studies demonstrated that the intact neuroendocrine response is critical to ensure survival and host defense mechanisms from secondary infectious challenges. In addition, our studies showed that acute activation of the hypothalamo-pituitary-adrenal (HPA) axis, SNS and endogenous opioid system impair counterregulatory responses to shock and trauma. In addition, we demonstrated that opiate analgesia disrupts the balanced neuroendocrine and opiate counterregulatory mechanisms that control hemodynamic, metabolic and pro-inflammatory responses to trauma/hemorrhage. The stress and analgesia induced disruptions in response exacerbate hemodynamic instability, compromise tissue perfusion and predispose to tissue injury and impaired innate host-defense response to a subsequent challenge. Taken together these findings indicate that stress-induced alteration in the neuroendocrine milieu of the host at the time of traumatic injury impacts immediate and long term mechanisms involved in restoration of homeostasis. Not only are the immediate cardiovascular mechanisms affected, but immune function appears to be impaired rendering the host more susceptible to morbidity and mortality from a secondary infection.

Final report on salient findings:

- a. ***Hemorrhage-induced β -endorphin release produces naltrexone-sensitive analgesia.*** The rise in circulating β -endorphin levels coincides with the establishment of an analgesic response as measured by the latency of the tail-flick response. This effect is sustained surpassing the time period during which β -endorphin levels are elevated and may be the result of synergy between β -endorphin and norepinephrine.
- b. ***Circulating and tissue levels of Substance P are not upregulated by trauma/hemorrhage.*** Several studies have suggested that neurokinins may contribute to the pro-inflammatory responses associated with trauma. We performed a time-course study to investigate whether a similar response to that of β -endorphin and norepinephrine could be detected following trauma/hemorrhage. No significant elevations in circulating levels of substance P were detected. Furthermore, no difference in crushed muscle content was noted, suggesting that either the turnover of the peptide is such that we are not able to detect modulation of its release by the available methodology or that its release in the peripheral tissues in response to trauma and shock is negligible, contributing little or nothing to modulation of the pro-inflammatory responses observed. Our results were confirmed in a model using a nociceptive stimulus (turpentine 0.6 ml IM). In contrast to what we expected, our results showed an overall decrease in circulating substance P values following turpentine injection. We concluded that in this conscious unanesthetized model of trauma/hemorrhage the contribution

of substance P as modulator of the early pro-inflammatory responses is probably of lesser importance to that of opioids and norepinephrine. The results from our studies on sensory denervated animals would lend support to this hypothesis.

c. *Impact of morphine analgesia on the outcome from trauma/hemorrhage.* Opiate analgesia is part of the mainstay approach to treatment of the wounded victim. Our studies have provided evidence that endogenous opioid peptides released during shock and trauma play a deleterious role on the hemodynamic and pro-inflammatory responses to shock and trauma. Our studies showed that a) morphine analgesia (10 mg/kg IP) prior to fluid resuscitation increased mortality from trauma/hemorrhage to 36% within the first 48 h following shock and trauma, b) morphine analgesia following trauma/hemorrhage impairs host response to a "second-hit" inflammatory challenge and c) morphine analgesia alters pressor response to fluid resuscitation which was later shown to result in increase fluid resuscitation volume to restore mean arterial pressure and d) Ketamine analgesia does not provide beneficial effects over those of morphine.

d. *Impact of time of surgery in relation to time of trauma on neuroendocrine responses.* The study of conscious and unrestrained animals for the investigation of the neuroendocrine regulation of hemodynamic and inflammatory responses requires that animals be surgically fitted with vascular catheters. Several investigators have proposed the need for a recovery period from the surgical procedure prior to performing the hemorrhage experiment. We investigated the impact of a one week recovery period on selected parameters. Two groups of animals were used; one was implanted with vascular catheters on the day prior to trauma/hemorrhage while the other group had catheters implanted one week before. Animals were handled throughout the week recovery period. Our results show that corticosterone, epinephrine and norepinephrine values were not different between groups at baseline and these did not differ in the magnitude of the response following trauma/hemorrhage. In addition, it appears that the immediate pro-inflammatory response that is elicited in the two groups of animals was not significantly different between the two groups.

e. *Development of a model to assess the impact of environmental stress on outcome from trauma/hemorrhage.* In the process of establishing and characterizing a reproducible stress model; we examined the impact of exercise under increased ambient temperature conditions (treadmill run to exhaustion; 0.24m/s, 6° grade, T: 38-39 °C) and that of restraint stress (60 min BID x 7 d) followed by tx/hem on the neuroendocrine response to traumatic injury. Our results showed that exercise and restraint stress produce differential cardiovascular

and neuroendocrine effects, with restraint stress but not exercise in high ambient temperatures producing significant elevations in resting MABP, epinephrine and norepinephrine levels compared to those of control animals. In addition, our results showed that exercise/heat stress did not have a marked impact on early hemodynamic responses to hemorrhage. There was a tendency for the exercise/stress animals to have lower mean arterial blood pressure. Overall, this is not considered to be of significant physiologic impact, as the immediate (post-fluid resuscitation) lung and spleen pro-inflammatory response (TNF & IL-1) to tx/hem were not affected in exercised stressed animals. Furthermore, restraint stress for 7 consecutive days prior to hemorrhagic shock did not produce significant alteration in the immediate hemodynamic counter regulatory responses to blood loss nor did it alter the response to fluid resuscitation. These findings led to the development and characterization of a model of physical and psychological stressed with immobilization described below. Our results indicate that immobilization stress is a more potent and reproducible activator of autonomic and neuroendocrine responses than restraint stress.

Previous publications from this grant:

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2. Meijerink WJ, Molina PE, Abumrad NN. Mammalian opiate alkaloid synthesis: lessons derived from plant biochemistry. *Shock.* 3:165-73, 1999.
3. Molina PE, Abumrad NN. Central sympathetic modulation of tissue cytokine response to hemorrhage. *Neuroimmunomodulation.* 3:193-200, 1999.
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6. Molina PE. Noradrenergic inhibition of stress-induced TNF upregulation in hemorrhagic shock. *J Neuroimmunomodulation.* 9:125-133, 2001.
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8. Phelan H, Stahls P, Hunt J, Bagby G, Molina PE. Impact of alcohol intoxication on hemodynamic, metabolic, and cytokine responses to hemorrhagic shock. *J Trauma.* 52(4): 675-82, 2002.

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10. Molina PE. Endogenous opioid analgesia in hemorrhagic shock. J Trauma. 54(5 Suppl):S126-32, 2003.
11. Molina PE, Zambell KL, Zhang P, Vande Stouwe C, Carnal J. Hemodynamic and immune consequences of opiate analgesia after trauma/hemorrhage. Shock. 21(6):526-34, 2004.
12. Molina PE. Neurobiology of the stress response: contribution of the sympathetic nervous system to the neuroimmune axis in traumatic injury. Shock. 24(1):3-10, 2005.